

Effect of Granulocyte Colony-Stimulating Factor Treatment at a Low Dose but for a Long Duration in Patients With Coronary Heart Disease

— A Pilot Study —

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Background In animal models, granulocyte colony-stimulating factor (G-CSF) improves post-infarct cardiac function. However, in pilot studies involving patients with angina and acute myocardial infarction (AMI), G-CSF at a high dose frequently induced coronary occlusion or restenosis, but those at a low dose showed no significant beneficial effect. We hypothesized that a low dose but long duration of G-CSF will have a beneficial effect without serious complications to patients with coronary heart disease.

Methods and Results Forty-six patients with angina or AMI were randomly assigned into G-CSF and non-G-CSF control groups, respectively. Recombinant G-CSF was subcutaneously injected once a day for 10 days. The leukocyte counts in the peripheral blood were controlled at approximately 30,000/ μ l. One month later, a Thallium-201 single photon emission computed tomography revealed the increased percentage uptake and the reduced extent and severity scores in the G-CSF angina group. In the G-CSF AMI group, the curve between the ejection fraction and peak creatine kinase shifted significantly upward, compared with that of the non-G-CSF AMI group. Serious complications were not observed during the 6 months of observation.

Conclusions A low dose but long duration of G-CSF treatment may have a beneficial effect without any serious complications in patients with coronary heart disease. (Circ J 2006; 70: 430–437)

Key Words: Acute myocardial infarction; Angina; Granulocyte colony-stimulating factor; Regeneration therapy; Thallium

Recently, repair-regeneration therapy using various cell transplantations, cytokines such as granulocyte colony-stimulating factor (G-CSF), and low molecules such as myelosuppressives, has been experimentally and/or clinically trialed for the treatment of cardiac diseases.^{1,2} In many experimental studies, the subcutaneous injection of G-CSF, a regeneration and repair-related cytokine, improved cardiac function and the remodeling of post-infarct hearts.^{3–7} In recent clinical pilot studies, the subcutaneous injection of 10 μ g/kg per day of filgrastim (Amgen, USA) for 4 or 5 days, when the number of leukocytes reached more than 50,000/ μ l, induced occlusive thrombi of the coronary arteries in 70% of patients with acute myocardial infarction (AMI)⁸ and in 20% of patients with intractable angina.⁹ In contrast, 5 μ g/kg per day of

filgrastim for 4 days in the case of AMI and 6 days for angina showed no improvement of blood perfusion in patients with angina pectoris,¹⁰ and no significant improvement of left ventricular (LV) function in patients with AMI,¹¹ although no serious complications were seen. Our hypothesis is that a low dose but long duration of G-CSF may improve myocardial blood flow and/or cardiac function without causing serious complications in patients with coronary heart disease. Thus, this clinical G-CSF pilot study was designed to test this hypothesis.

Methods

Patient Population

We obtained written informed consent from all subjects after the ethics committee of our university approved this study. The data was controlled and estimated by an independent organization (Center for Medical Welfare Support in Gifu University Hospital). Forty-six patients aged less than 80 years were enrolled. The 22 patients with angina had viable but ischemic myocardial regions detected by stress-redistribution Thallium-201 (²⁰¹Tl) single photon emission computed tomography (SPECT), and were not eligible for revascularization therapy on the basis of coronary angiography (CAG). The other 24 patients had first AMI with single vessel disease, in which the infarct-related artery was the proximal left anterior descending coronary

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Table 1 Characteristics of Patients

	Control group	G-CSF group	p value
Angina			
No. patients	11	11	NS
Age	63±10	65±9	NS
Gender (M:F)	8:3	7:4	NS
Smoking	2	4	NS
No. diseased vessels	2.7±0.5	2.5±0.5	NS
Complications			
OMI	8	6	NS
CABG	4	6	NS
HTN	9	8	NS
HL	9	8	NS
DM	6	6	NS
Conventional therapy			
β-blocker	1	2	NS
ACE inhibitor	4	5	NS
ARB	3	1	NS
Ca ²⁺ channel blocker	9	7	NS
Statin	8	6	NS
AMI			
No. patients	12	12	NS
Age	65±9	62±11	NS
Gender (M:F)	10:2	8:4	NS
Culprit lesion (LAD proximal)	12	12	NS
Time to reperfusion (min)	194±85	229±96	NS
Pre-infarct angina (Yes:No)	6:6	6:6	NS
Peak CK (IU/L)	4,868±2,832	5,028±2,447	NS
Stent/POBA	6:6	8:4	NS
Stenosis rate			
Immediately after direct PCI (0/25/50/75/>90)	5/6/1/0/0	5/6/1/0/0	NS
1 month (0/25/50/75/>90)	0/1/1/0/0	1/10/0/1/0	NS
Complications			
HTN	6	5	NS
HL	4	5	NS
DM	4	3	NS
Conventional therapy			
β-blocker	2	2	NS
ACE inhibitor	3	4	NS
ARB	4	4	NS
Ca ²⁺ channel blocker	5	4	NS
Statin	4	5	NS

G-CSF, granulocyte colony-stimulating factor; OMI, old myocardial infarction; CABG, coronary artery bypass grafting; HTN, hypertension; HL, hyperlipidemia; DM, diabetes mellitus; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; AMI, acute myocardial infarction; LAD, left anterior descending; CK, creatine kinase; POBA, plain old balloon angioplasty; PCI, percutaneous coronary intervention.

artery and was successfully recanalized using direct percutaneous coronary intervention (PCI). The peak creatine kinase (CK) was determined by the measurements at 3-h intervals. Patients with severe heart failure (ejection fraction of less than 20%), and/or PCI within the previous 3 months, history of cancer, evidence of proliferative retinopathy, critical stenosis of carotid artery, interstitial pneumonia, active collagen disease, pregnancy or other severe concurrent illness were excluded from the present study.

Procedures

The 22 angina and 24 AMI patients were randomly assigned to a G-CSF or non-G-CSF control group, respectively. Treatment with antiplatelets (aspirin: 81 mg) and anticoagulants (warfarin: prothrombin time international normalized ratio of approximately 2.0) was started for all patients, and was continued during the study. All patients were treated with conventional drug therapies, as shown in Table 1. In the G-CSF groups, the subcutaneous injection of recombinant human G-CSF (lenograstim; Chugai Pharmaceutical Co Ltd, Japan) was performed once a day for 10 days. In the AMI G-CSF group, G-CSF started 2–4

days after the onset of AMI and was injected daily for 10 days. The white blood cell (WBC) count in the peripheral blood was measured every day. Lenograstim at 2 µg/kg per day was injected initially and the next dose was increased or decreased such that the WBC count reached approximately 30,000/µl. CD34-positive cells in the peripheral blood were measured before and 7 days after the treatment. Chest and abdominal computed tomography were performed to evaluate splenomegaly and interstitial pneumonia 5 days after starting the G-CSF therapy.

Before the treatment and 1 month later, ²⁰¹Tl SPECT imaging, electrocardiograms (ECG) Gated ^{99m}Tc-sestamibi imaging and CAG were performed in the angina group, and left ventriculography and CAG were performed in the AMI group. The clinical condition in each patient was followed up for 6 months. Each of these measurements was performed by 2 experienced persons blinded to the conditions.

²⁰¹Tl SPECT Imaging

Stress was induced by exercise using a bicycle ergometer or dipyridamole. Exercise with an ergometer was performed with an initial level of 25 W, which was increased by 25 W

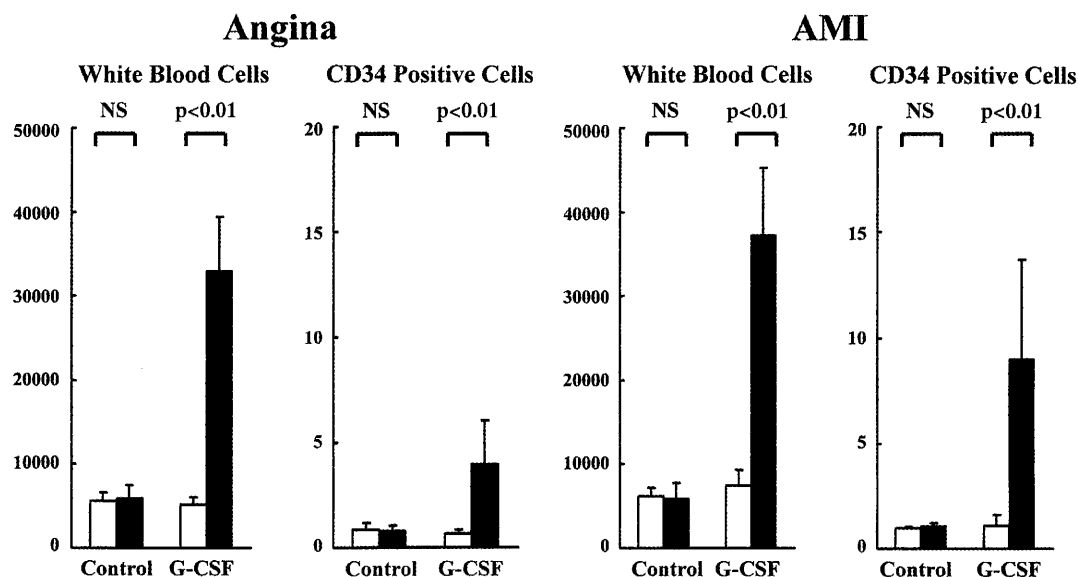


Fig 1. White blood cell (WBC) counts and CD34-positive cells in the peripheral blood. WBC counts and CD34-positive cells are markedly increased after granulocyte colony-stimulating factor (G-CSF) treatment in both the angina and acute myocardial infarction (AMI) groups. Note the greater WBC counts and CD34-positive cells before G-CSF treatment in the AMI group, which were measured 3 days after the onset of AMI, than those of the angina group. □, before treatment; ■, 7 days after starting treatment.

every 3 min. The cardiac rhythm was monitored continuously, 12-lead ECG were acquired every 3 min, and the arterial blood pressure was measured at baseline and at the end of each workload. At the near-maximal peak of exercise, 111 MBq ^{201}Tl was injected, and exercise was continued for another minute. In contrast, dipyridamole was administered intravenously at a rate of 0.56 mg/kg of bodyweight for 4 min. ^{201}Tl (111 MBq) was injected 8 min after the beginning of dipyridamole infusion. Initial images were obtained 10 min after ^{201}Tl injection, and delayed images were obtained 3 h later. It should be emphasized that the extent of loaded exercise and the dose of injected dipyridamole were identical between the pre- and post-treatment studies in each patient. SPECT images were acquired by using a 3-detector gamma camera (PRISM3000XP, Picker, Cleveland, OH, USA), which has a low-energy, high-resolution collimator. Signals were obtained from a 20% symmetrical window at 75 keV using the step-and-shoot acquisition method with 5-degree intervals and a 30-s dwell time per stop.

Analysis of the ^{201}Tl Imaging

Each short axis slice was divided into 60 sectors of 6 degrees each, and a bull's-eye map was reconstructed from the short axis slices extending from the base to the apex. The pixel with the maximal count was selected, and all pixel counts were normalized to a maximal count of 100. The bull's-eye map was divided into 8 segments and the percentage uptake of each segment was calculated. Each bull's-eye pixel in the patient's file was compared with the corresponding pixels in the file obtained from 15 normal volunteers; pixels that exceeded 2 SD below the limit of normal were defined as abnormal. The extent score was the sum of abnormal points, and the severity score was the sum of the difference between the count of abnormal pixels and those of the corresponding normal pixels/total number of all pixels.

ECG Gated $^{99\text{m}}\text{Tc}$ -Sestamibi Imaging

Resting ECG gated $^{99\text{m}}\text{Tc}$ -sestamibi images were reconstructed in the G-CSF treatment group before and 4 weeks after the therapy. One hour after injection of 600 MBq of $^{99\text{m}}\text{Tc}$ -sestamibi, SPECT acquisition was started. Data acquisition was performed in a similar manner to ^{201}Tl SPECT imaging. Signals were gated at 16 frames per cardiac cycle. LV end-diastolic volume (LVEDV), end-systolic volume, and ejection fraction (LVEF) were automatically calculated with quantitative gated SPECT software (QGS, Cedars-Sinai Medical Center, Los Angeles, CA, USA) on a Picker workstation.¹²

LV Cineangiography

Left ventriculography was performed with the use of a 4 Fr pigtail catheter proceeded through the radial artery. Projection images were acquired in 2 directions (30° right anterior oblique view and 60° left anterior oblique view) as previously detailed.¹³

Statistical Analysis

All measures were expressed as mean \pm SD. Statistical significance was defined as $p < 0.05$. Discrete variables were compared as rates, and comparisons were made by c-square analysis. Intra-individual comparison of continuous variables was performed by using the paired t-test. Differences in each parameter between the control and G-CSF groups were assessed by using the unpaired t-test.

Results

Patient Characteristics

As shown in Table 1, significant differences were not seen between the G-CSF and non-G-CSF groups with angina or AMI in terms of age, gender, number of diseased vessels, past history, risk factors and conventional therapy.

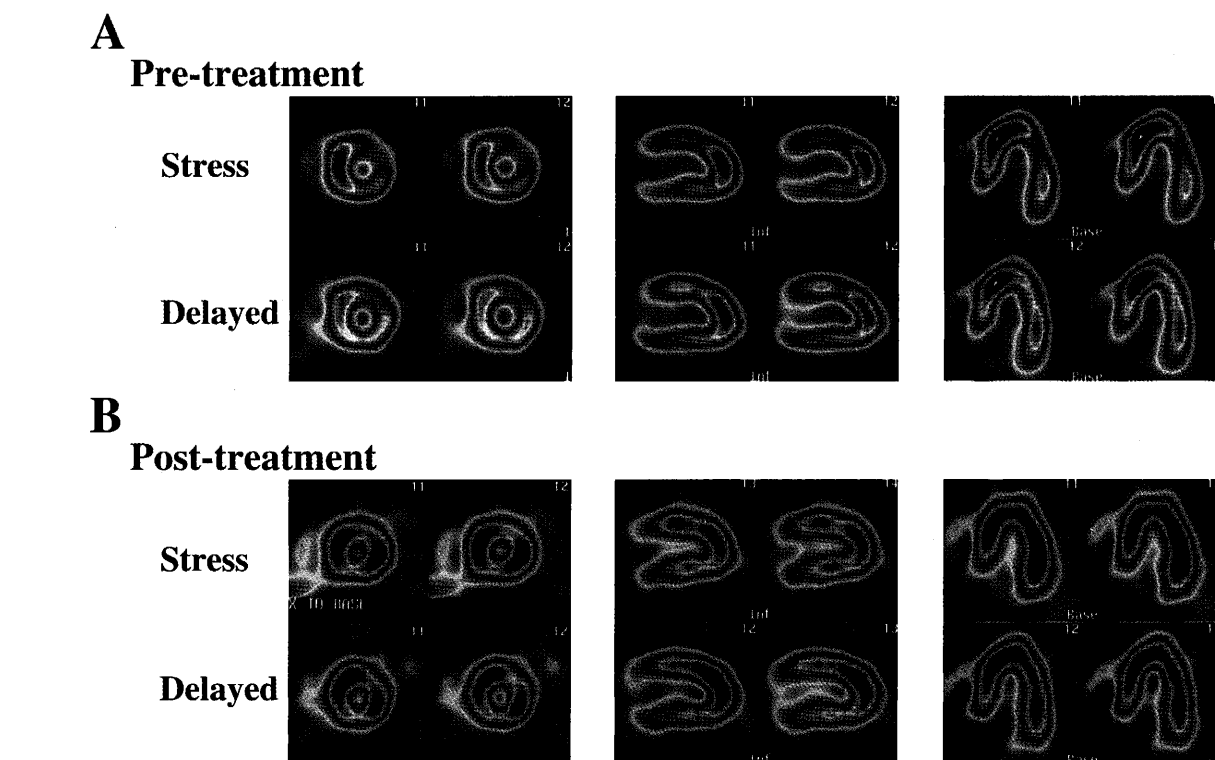


Fig 2. Thallium-201 (^{201}Tl) single photon emission computed tomography imaging of a 55-year-old man with intractable angina pectoris treated with granulocyte colony-stimulating factor (G-CSF) who has 3 vessel coronary heart disease in spite of several percutaneous coronary intervention and a low ejection fraction of 25% and chronic renal failure. Note the defect of ^{201}Tl in the anterolateral wall before G-CSF treatment and the improvement after the treatment.

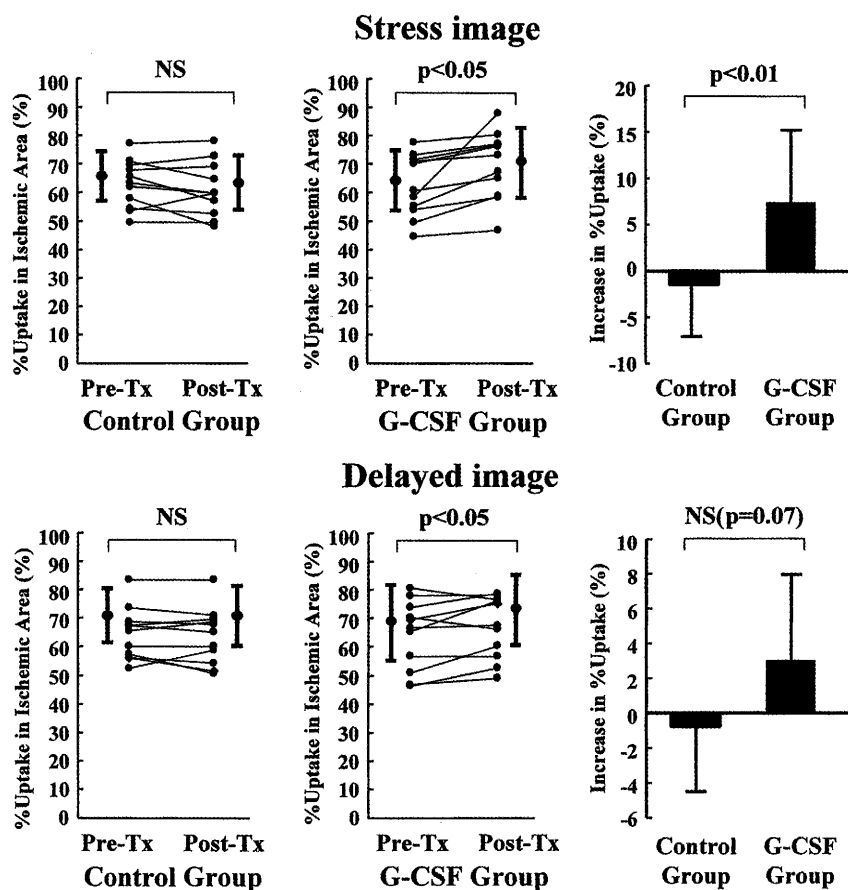


Fig 3. Percentage uptake on stress and delayed images in the ischemic area by Thallium-201. Note the significant increases of the percentage uptake in the ischemic area 1 month after granulocyte colony-stimulating factor (G-CSF) treatment (Tx).

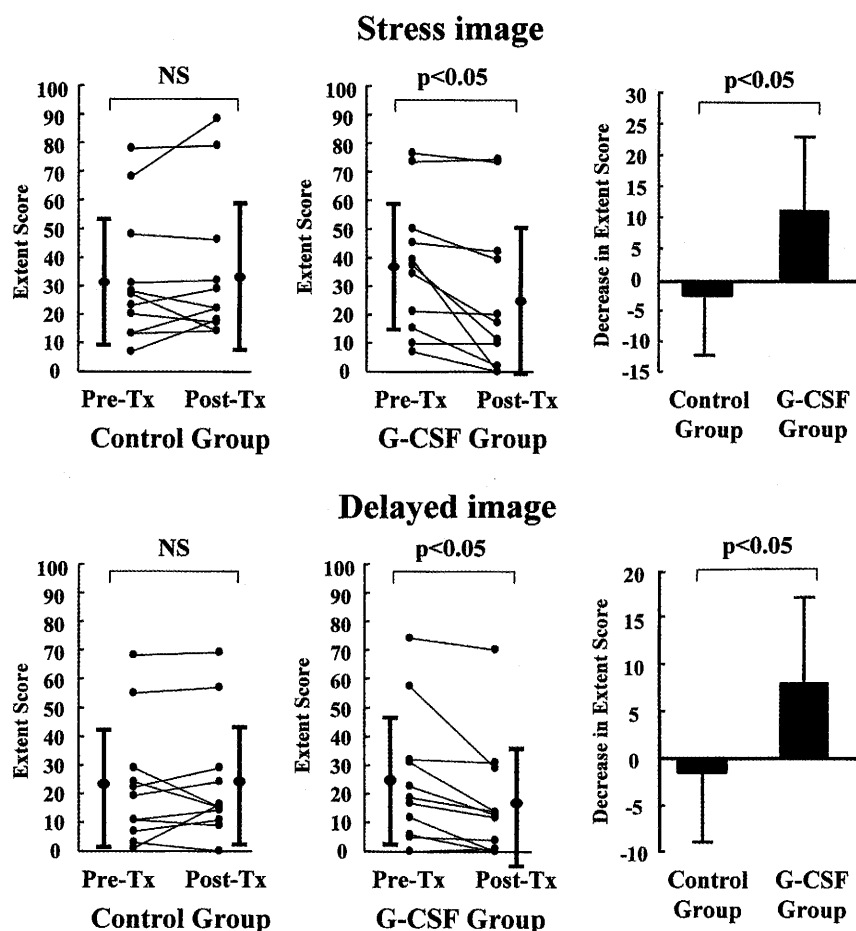


Fig 4. Extent scores of the stress and delayed Thallium-201 images. Note the significant decrease of the extent scores 1 month after granulocyte colony-stimulating factor (G-CSF) treatment (Tx).

General Conditions

During the 6-month follow up, there were no serious complications such as death, acute coronary syndrome, cerebral infarction, splenomegaly or interstitial pneumonia in either the G-CSF or non-G-CSF group.

CAG Findings

There was no progression of coronary arterial stenosis according to CAG between that before and 1 month after the treatment in each patient of the angina groups. In the AMI group, the restenosis rate of the infarct-related coronary arteries after direct PCI was similar between the G-CSF AMI group (1 of 12 patients) and the non-G-CSF AMI group (0 of 12 patients).

Dose of G-CSF, Peripheral WBC Counts and CD34 Positive Cells

In the G-CSF angina group, the dose of G-CSF was $3.1 \pm 0.8 \mu\text{g/kg}$ per day. The number of WBC in the peripheral blood was increased from $5,118 \pm 960/\mu\text{l}$ before treatment to $32,882 \pm 6,415/\mu\text{l}$ after treatment (Fig 1). The number of CD34 positive cells in the peripheral blood was $0.6 \pm 0.2/\mu\text{l}$ before treatment and increased to $3.9 \pm 2.1/\mu\text{l}$ after the therapy (Fig 1). However, there was no significant increase of these parameters in the non-G-CSF group.

In the G-CSF AMI group, the dose of G-CSF was $2.6 \pm 0.5 \mu\text{g/kg}$ per day. The number of WBC in the peripheral blood increased from $7,520 \pm 1,830/\mu\text{l}$ before G-CSF treatment to $37,218 \pm 8,045/\mu\text{l}$ after treatment. The number of CD34-positive cells increased from $1.1 \pm 0.5/\mu\text{l}$ at pretreat-

ment to $9.0 \pm 4.7/\mu\text{l}$ after treatment (Fig 1). However, the number did not increase significantly in the non-G-CSF group.

The increase of WBC consisted of those of granulocytes and monocytes in each of the angina and AMI groups. There was no significant change in the platelet, red blood cell or lymphocyte counts in the peripheral blood after G-CSF treatments.

^{201}Tl Scintigraphy in Angina Groups

In the angina groups, the percentage uptake on stress images by ^{201}Tl in the ischemic area increased significantly after G-CSF treatment ($63 \pm 11\%$ to $71 \pm 12\%$, respectively) 1 month later, but did not change in the non-G-CSF group (Figs 2,3). Extent scores on the stress and delayed ^{201}Tl images decreased significantly in the G-CSF group (37 ± 23 to 26 ± 27 and 25 ± 23 to 17 ± 21 , respectively), although significant changes were not observed in the non-G-CSF group (Fig 4). Similarly, severity scores decreased significantly in the G-CSF group on the stress and delayed images (51 ± 55 to 33 ± 46 and 31 ± 44 to 23 ± 38 , respectively), but there was no significant change in the non-G-CSF group (Fig 5).

LV Function at Rest by ECG Gated ^{99m}Tc -Sestamibi Imaging in Angina Groups

In each of the G-CSF and non-G-CSF angina groups, there was no significant alteration in LVEDV, LVEF or regional wall motion between that before and 1 month after the study.

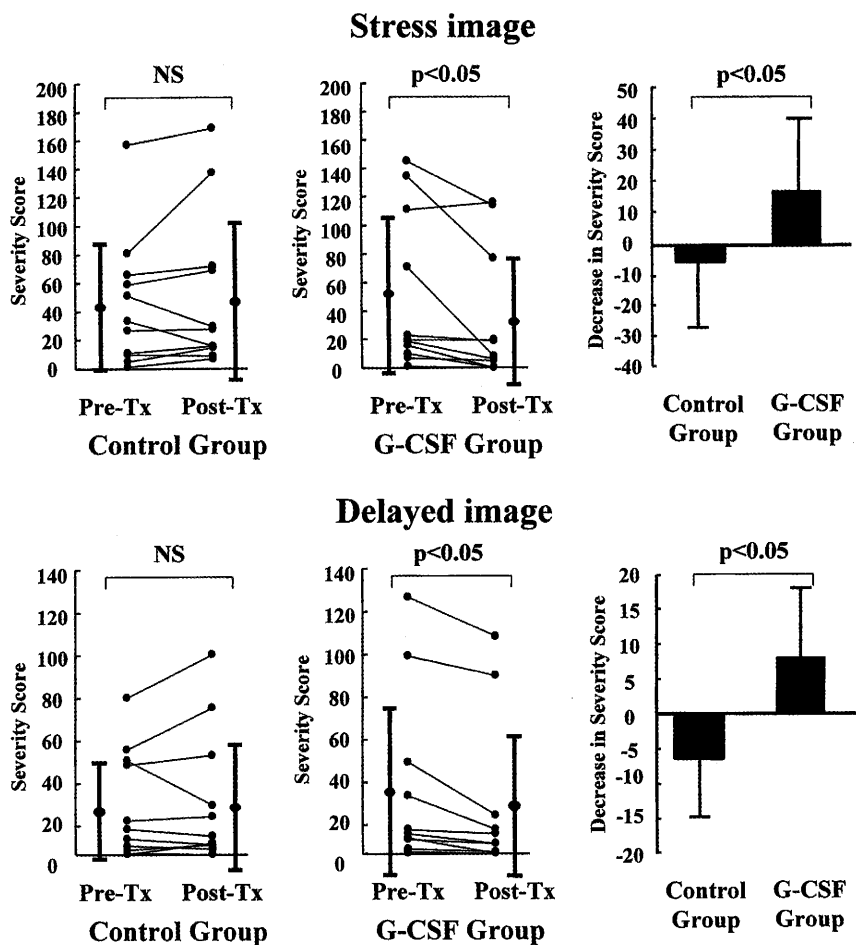


Fig 5. Severity scores of the stress and delayed Thallium-201 images. Note the significant decrease of the severity scores 1 month after granulocyte colony-stimulating factor (G-CSF) treatment (Tx).

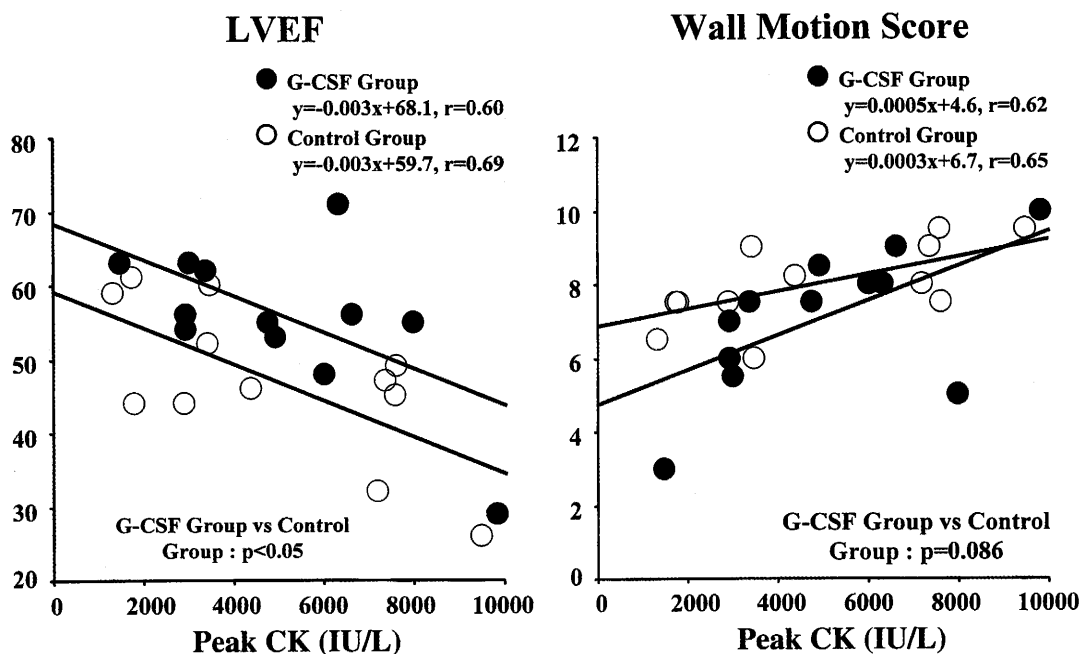


Fig 6. Ejection fraction and regional wall motion score 1 month after the onset of infarction according to left cineangiography in the acute myocardial infarction (AMI) groups. Note the shift of the curve between the ejection fraction and the peak creatine kinase (CK) to the upward in the granulocyte colony-stimulating factor (G-CSF) AMI group from that of the non-G-CSF AMI group. The regional wall motion score showed a tendency for improvement in the G-CSF group, although the difference was not significant. LVEF, left ventricular ejection fraction.

LV Function by LV Cineangiography in AMI Groups

In the AMI groups, the curve between the ejection fraction at 1 month after the onset and the peak CK in the G-CSF AMI group shifted significantly upward compared with that of the non-G-CSF AMI group (Fig 6). However, regional wall motion score and LVEDV at 1 month after the onset showed no significant differences between the G-CSF and non-G-CSF groups, although the regional wall motion score of the G-CSF AMI group showed a downward tendency ($p=0.086$) compared with that for the non-G-CSF AMI group, as shown in Fig 6.

Discussion

Ameliorating Effect of G-CSF Therapy

In the angina group, G-CSF of lenograstim increased the percentage uptake and reduced the extent and severity scores in ischemic regions on the ^{201}Tl image, indicating the improvement of myocardial ischemia; that is, the increase of regional blood flow. Our result using lenograstim ($3.1\mu\text{g/kg}$ per day $\times 10$ days) is different from that of a recent study showing that G-CSF of filgrastim ($5\mu\text{g/kg}$ per day $\times 6$ days) has no beneficial effect on patients with severe chronic ischemic heart disease.¹⁰ The total dose of G-CSF was similar between the 2 studies; however, it is well-known that various biological activities are higher in lenograstim than filgrastim in animals and humans.¹⁴ For example, using equal doses of the 2 products, the level of CD34-positive cells in humans was 27% more with lenograstim than filgrastim. To have a similar reduction of neutropenia with chemotherapy in humans, it was necessary to use $5\mu\text{g/kg}$ of filgrastim compared to only $3.5\text{--}4\mu\text{g/kg}$ of lenograstim. Thus, the difference between the lenograstim and filgrastim studies may be explained by the higher biological activity of lenograstim rather than the longer duration of the dose.

In the AMI group, lenograstim ($2.6\mu\text{g/kg}$ per day $\times 10$ days) significantly improved LVEF at 1 month after AMI; however, there was no significant difference in the data found by Valgimigli et al (filgrastim: $5\mu\text{g/kg}$ per day $\times 4$ days), although a tendency towards improvement was seen;¹¹ that is, the total dose was higher than theirs. In addition, the higher biological activity of lenograstim as detailed above¹⁴ may be an important cause of the difference. Thus, the improvement of LVEF in the present study may depend on the higher total dose and higher biological activity of lenograstim rather than on the longer duration of the dose.

In the angina group, G-CSF did not improve LV functions at rest. Generally, it is difficult to detect the improvement of LV function that is already nearly normal before treatment at rest, such as in the present study patients. Therefore, for the comparison of LV function, stress may be needed for the detection. This is supported by the fact that the improvements of the ^{201}Tl accumulation in the ischemic myocardium were more prominent on the stress images than the delayed images.

Possible Mechanism of the Beneficial Effects

In the angina group, G-CSF increased regional blood flow in the ischemic myocardium. CD34-positive cells, precursor cells of vascular endothelial cells, smooth muscle cells and cardiomyocytes,¹⁵ in the peripheral blood were markedly increased in the G-CSF-treated group; however, 1 month later, CAG itself showed no alteration. Therefore,

the improvement of blood flow may be caused by the development of microvessels that are undetectable by conventional CAG.

In the AMI group, LVEF improved, and 2 possible mechanisms were considered: (1) the regeneration of myocardial tissues, because CD34-positive cells, precursor cells of various myocardial cells, in the peripheral blood were markedly increased; and (2) a paracrine effect such as the upregulation of matrix-metalloproteinases and/or a direct effect on cardiomyocytes via the activation of the Stat 3 receptor.^{5,16} Recent experimental studies including ours showed that the regeneration of cardiomyocytes in infarcted myocardial tissues by G-CSF may be too small to explain the improvement of LV function.^{5,16} In the present study, regional wall motion in the LV wall with infarction was not improved, suggesting that improvement in the LVEF may be caused by the effect of G-CSF on the LV wall without infarction as well as with infarction. In addition, in patients with successful direct PCI, myocardial ischemia is generally considered to not be present at the chronic stage, which was confirmed by the CAG findings 1 month after the onset of AMI in the present study. Therefore, the main mechanism of improved LVEF by G-CSF in these AMI patients may be the second rather than the first mechanism. Further investigation of the mechanism is warranted.

Difference of WBC, G-CSF Dose and Circulating CD34-Positive Cells Between Angina and AMI Groups

In this study, the dose of G-CSF was controlled at approximately $30,000/\mu\text{l}$ of the WBC count in the peripheral blood for the protection of thrombo-embolism; however, the WBC count before G-CSF treatment (3 days after the onset of AMI in the AMI group) was greater in the AMI group than in the angina group (Fig 1). This would explain why the dose of G-CSF was smaller in the AMI group than in the angina group.

In contrast, circulating CD34-positive cells before G-CSF treatment were greater in the AMI group than in the angina group (Fig 1). In addition, the increased rate after G-CSF treatment was larger in the AMI group in spite of the smaller dose of G-CSF. This suggests a higher response of bone marrow stem cell mobilization into the peripheral blood in AMI than angina.

No Serious Side Effects of G-CSF Treatment

It has been reported that G-CSF facilitates platelet aggregation,^{17,18} and the injection of $10\mu\text{g/kg}$ per day filgrastim for 4 or 5 days in patients with angina pectoris and AMI induced serious complications of restenosis after PCI and acute coronary syndrome.^{8,9} Similar life-threatening complications by G-CSF injection, including myocardial and cerebral infarction and spontaneous rupture of the spleen, have already been reported in peripheral blood stem cell transplantation in healthy humans.^{9,20} The daily injected dose of G-CSF is usually 5 to $10\mu\text{g/kg}$, and the peripheral WBC counts frequently exceed $50,000/\mu\text{l}$. Therefore, in the present study, the lower dose and longer duration of lenograstim ($3.1\mu\text{g/kg}$ per day $\times 10$ days in the angina group; $2.6\mu\text{g/kg}$ per day $\times 10$ days in the AMI group) under antiplatelet agents and anticoagulants were examined and WBC counts were controlled to a level that was approximately $30,000/\mu\text{l}$. There were no serious side-effects of the lenograstim treatment, such as acute coronary syndrome, cerebrovascular disease, spleen rupture or other serious diseases during the 6-month observation period. The lack

of serious side-effects was similar to recent studies using 5 µg/kg per day filgrastim for 6 days for angina and for 4 days for AMI^{10,11}. This supports the safe use of lenograstim used in the present study.

Study Limitation

The present study trial was not designed as a placebo-controlled study because the frequent occurrence of painful bones makes G-CSF a potential unblinding agent. In fact, approximately one-third of patients receiving lenograstim in the present study experienced painful bones, even though we used only a low dose of the drug. Also, the present pilot study is limited by the small number of participating patients. Therefore, a clinical trial with a larger number of patients is warranted for the establishment of the ameliorating effect and the safety of G-CSF.

Conclusion

Careful lenograstim injection (3.1 µg/kg per day × 10 days in the angina group; 2.6 µg/kg per day × 10 days in the AMI group) combined with anti-platelet agents and anti-coagulants may be effective and safe to use in the treatment of Japanese patients with coronary heart disease.

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References

1. Fukuda K. Progress in myocardial regeneration and cell transplantation. *Circ J* 2005; **69**: 1431–1446.
2. Misao Y, Arai M, Ohno T, Ushikoshi H, Takahashi T, Takemura G, et al. Cyclophosphamide improves the function of post-infarct hearts by reducing old infarct area and accelerating the mobilization of CD34⁺ cells. *Circ J* 2005; **69**: 763–765.
3. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad USA* 2001; **98**: 10344–10349.
4. Ohtsuka M, Takano H, Zou Y, Toko H, Akazawa H, Qin Y, et al. Cytokine therapy prevents left ventricular remodeling and dysfunction after myocardial infarction through neovascularization. *FASEB J* 2004; **18**: 851–853.
5. Minatoguchi S, Takemura G, Chen XH, Wang N, Uno Y, Koda M, et al. Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by postinfarction granulocyte colony-stimulating factor treatment. *Circulation* 2004; **109**: 2572–2580.
6. Iwanaga K, Takano H, Ohtsuka M, Hasegawa H, Zou Y, Qin Y, et al. Effects of G-CSF on cardiac remodeling after acute myocardial infarction in swine. *Biochem Biophys Res Commun* 2004; **325**: 1353–1359.
7. Sugano Y, Anzai T, Yoshikawa T, Maekawa Y, Kohno T, Mahara K, et al. Granulocyte colony-stimulating factor attenuates early ventricular expansion after experimental myocardial infarction. *Cardiovasc Res* 2005; **65**: 446–456.
8. Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilized with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: MAGIC cell randomized clinical trial. *Lancet* 2004; **363**: 751–756.
9. Hill JM, Paul JD, Powell TM, McCoy JP, Dunbar CE, Horne M, et al. Efficacy and risk of granulocyte colony stimulating factor administration in patients with severe coronary artery disease (abstract). *Circulation* 2003; **108**(Suppl IV): 478.
10. Wang Y, Tägil K, Ripa RS, Nilsson JC, Carstensen S, Jørgensen E, et al. Effect of mobilization of bone marrow stem cells by granulocyte colony stimulating factor on clinical symptoms, left ventricular perfusion and function in patients with severe chronic ischemic heart disease. *Int J Cardiol* 2005; **100**: 477–483.
11. Valgimigli M, Rigolin GM, Cittanti C, Malagutti P, Curello S, Percoco G, et al. Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: Clinical and angiographic safety profile. *Eur Heart J* 2005; **26**: 1838–1845.
12. Germano G, Kiat H, Kavanagh PB, Moriel M, Mazzanti M, Su HT, et al. Automatic quantification of ejection fraction from gated myocardial perfusion SPECT. *J Nucl Med* 1995; **36**: 2138–2147.
13. Noda T, Minatoguchi S, Fujii K, Hori M, Ito T, Kanmatsuse K, et al. Evidence for the delayed effect in human ischemic preconditioning: Prospective multicenter study for preconditioning in acute myocardial infarction. *J Am Coll Cardiol* 1999; **34**: 1966–1974.
14. Höglund M, Smedmyr B, Bengtsson M, Tötterman TH, Cour-Chabernaud V, Yver A, et al. Mobilization of CD34⁺ cells by glycosylated and nonglycosylated G-CSF in healthy volunteers—a comparative study. *Eur J Haematol* 1997; **59**: 177–183.
15. Yeh ET, Zhang S, Wu HD, Koerbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34⁺-enriched cell population into cardiomyocytes, endothelial cells and smooth muscle cells in vivo. *Circulation* 2003; **108**: 2070–2073.
16. Harada M, Qin Y, Takano H, Minamino T, Zou Y, Toko H, et al. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. *Nat Med* 2005; **11**: 305–311.
17. Shimoda K, Okamura S, Inaba S, Okamura T, Ohga S, Ueda K, et al. Granulocyte colony-stimulating factor and platelet aggregation. *Lancet* 1993; **341**: 633.
18. Falanga A, Marchetti M, Evangelista V, Manarini S, Oldani E, Giovanelli S, et al. Neutrophil activation and hemostatic changes in healthy donors receiving granulocyte colony-stimulating factor. *Blood* 1999; **93**: 2506–2514.
19. Falzetti F, Aversa F, Minelli O, Tabilio A. Spontaneous rupture of spleen during peripheral blood stem-cell mobilization in a healthy donor. *Lancet* 1999; **353**: 555.
20. Becker PS, Wagle M, Matous S, Swanson RS, Pihan G, Lowry PA, et al. Spontaneous splenic rupture following administration of granulocyte colony-stimulating factor (G-CSF): Occurrence in an allogeneic donor of peripheral blood stem cells. *Biol Blood Marrow Transplantation* 1997; **3**: 45–49.